Chapter 9

Summary, discussion and future perspectives

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

In the last decades the survival of children with cancer has increased substantially, mainly due to more intensive treatment protocols. The drawback of the success is a tremendous increase in the side effects of the treatment [1,2]. One of the major side effects of chemotherapeutic treatment, affecting both the quality of life as well as causing a suboptimal treatment, is gastrointestinal mucositis, further referred to as mucositis. Mucositis is a clinical problem specifically in pediatric cancer patients caused by treatment protocols with high doses of chemotherapy. Children suffering from mucositis have symptoms like nausea, vomiting, abdominal pain, and diarrhea, leading to a decreased nutritional intake and a higher susceptibility to develop a bacteremia or sepsis [3-5]. These children are in need of nutritional support and high doses of pain medication to overcome these days of suffering. Side effects, like mucositis, may lead to a decrease in chemotherapeutic dosage, a delay of the next chemotherapeutic cycle, or even a discontinuation of the next chemotherapeutic cycle. Currently, cancer treatment is focusing on more targeted therapies. However, we do not know the effect of these targeted therapies on the side effects [6]. In this thesis we present studies, which aimed to improve the management of chemotherapy-induced mucositis in pediatric cancer patients. These studies can be categorized into two parts: 1) new insights into preventive and therapeutic agents for gastrointestinal mucositis; 2) novel information about the clinical care concerning risk, diagnosis and feeding strategy during gastrointestinal mucositis.

PREVENTION AND TREATMENT OF MUCOSITIS

The 5-phase model of the pathophysiology of oral mucositis was adapted for gastrointestinal mucositis. However, the exact pathophysiology has not been completely elucidated. It is thought that there is direct DNA damage caused by chemotherapy or radiotherapy. After this damage there is a cascade of events with activation of transcription factors, like NF-κb, and consequently induction of pro-inflammatory cytokines, like TNF-α. This will cause inflammation and cell death in the intestine, eventually resulting in ulcers which are portal entries for bacteria and fungi. Finally, it is a self-healing condition [4,7-9]. However, much of the pathophysiology is still unknown. All different phases in this model offer a potential target to alter the severity of mucositis. Since we have a validated mucositis rat model, where we induce mucositis with MTX, we had the possibility to determine the effect of interventions in the different phases of the pathophysiological model to see if this was a possible option to prevent or treat mucositis [10].

Promoting intestinal growth

The first phase of mucositis is the direct damage caused by the chemotherapeutic agent. Therefore, the first intervention of interest is either to prepare the intestine to be more resistant to the chemotherapy, or to speed up the recovery after the damage. Currently, for oral mucositis there is one approved therapy; Palifermin, a keratinocyte growth factor [11]. Palifermin stimulates proliferation and differentiation of epithelial cells and functions therefore as an epithelial growth factor [11]. Palifermin is only approved for patients with hematological malignancies receiving total-body irradiation-based chemotherapy followed by hematopoietic stem cell transplantation [11-13]. Unfortunately, there are conflicting results concerning the efficacy in mucositis induced by other chemotherapy regimens, and in less severe mucositis [11,12,14]. Even more, Palifermin did not prevent intestinal mucositis [15]. Therefore, searching for another growth factor of intestinal epithelial cells would be of interest to either protect or repair the intestine. From different animal models concerning other intestinal diseases like inflammatory bowel disease or total parenteral nutrition associated atrophy, oral insulin was shown to be a possible intestinal growth factor [16-19]. It was shown to have positive effects on histology in another, less severe, mucositis rat model [20]. Different local effects were seen in previous studies possibly due to differences between rapidly proliferating mucosa, for example after bowel resection or intestinal injury, and normal proliferation mucosa in healthy and diabetic rats [21]. Although the intestine is not the primary target of insulin, and insulin is broken down in the acidic environment of the stomach, there were insulin receptors found on the luminal and basolateral membrane of the enterocyte [22]. Therefore, it was suggested that oral insulin might have an effect in the intestine. We determined the effect of oral insulin in our previously established mucositis rat model in *chapter 2*. We administered oral insulin prior to and during mucositis to see both the effect on the severity as well as on the recovery phase. First, the data show that, regardless of the proliferation state of the mucosa of the small intestine, oral insulin did not influence the enterocyte mass in this mucositis rat model, measured with plasma citrulline. Furthermore, oral insulin did not influence any clinical parameter, histology or intestinal function after MTX. Therefore, from *chapter 2* we conclude that oral insulin does not influence mucositis induced by MTX in the rat, and we conclude that in our rat model this was not an effective prevention or treatment and it is not useful to further study this intervention. However, another intestinal epithelial growth factor might be of interest to either protect the intestine from damage and thereby decrease the severity of mucositis, or to repair the intestine and thereby fasten the recovery, for which more research is needed.

Blocking pro-inflammatory cytokines

In the five-phase model the pro-inflammatory cytokines seem to be key players and are therefore another possible target option for prevention or treatment of mucositis [23]. Moreover, in several

studies in mucositis models it was shown that an inhibitor of a pro-inflammatory cytokine, like tumor necrosis factor-alpha (TNF-α), is effective in decreasing mucositis [24,25]. For that reason, we aimed to determine the effect of the TNF-α inhibitor Etanercept in our previously established mucositis rat model in *chapter 3*. We administered the TNF-α inhibitor on a daily basis prior to and during mucositis. This would decrease the mucosal inflammation after MTX injection, which, based on the pathophysiological model, would decrease the inflammation which should alter the severity of mucositis. However, the results show that this TNF-α inhibitor did not influence the clinical parameters, the intestinal damage and inflammation. Furthermore, TNF-α inhibitor did not influence the function of the intestine, and finally it did not influence the clinical parameters and histology in the recovery phase. Therefore, we conclude in *chapter 3* that a TNF-α inhibitor alone is not effective in decreasing the severity of mucositis if it is induced by high dose MTX in the rat. However, we suggest that a TNF-α inhibitor might still be of interest if it is combined with other interventions. We conclude that TNF-α is not the key factor in the pathophysiology of mucositis. A possible explanation is that mucositis is so complex and therefore it is not possible to alter the severity by interfering with only one target. However, even if mucositis is such a complex inflammatory process, the inhibition of a major pro-inflammatory cytokine should alter the severity at least at some level. Therefore, we can speculate that the pathophysiology is different than we thought. Inflammation might be a secondary or even a separate pathway that is also present during mucositis, but not the main cause of damage. This would cause a different focus of targeting and influencing the severity and more research is needed.

Influencing bile salt synthesis

Looking again at the potential pathophysiological model, so far all studied prevention and treatment strategies targeted only one phase in this model. However, it would be of potential interest if an intervention would focus on several factors of the pathophysiological model.
Bile salts are influenced by chemotherapy and important in several gastrointestinal disorders [26-28] The amount and composition of bile salts are important in the intestine, possibly causing toxicity, diarrhea and bacterial overgrowth [29-32]. Bile salts regulate their own synthesis via activation of the Farnesoid X receptor (FXR). The FXR is a nuclear receptor present in, among other things, the enterocytes of the small intestine, directly interacting with DNA and control transcription [30,33]. Activated FXR in the enterocyte decreases bile salt synthesis in the liver [34]. In other intestinal animal models, like inflammatory bowel disease and total parenteral nutrition associated atrophy, the FXR and thereby the agonists bile salts, seems to be of influence on both the severity as well as the recovery [35-39]. Furthermore, FXR-agonists potentially influenced microbiota and improved villus length and crypt length [35-39]. Targeting FXR, and thereby bile salts, might interfere in multiple targets of the 5-phase model of mucositis in a beneficial way. Therefore, in *chapter 4* we determined the effect of an FXR-agonist on the severity

and recovery of MTX-induced mucositis in rats. Unexpectedly, the results show that the administration of an FXR-agonist directly prior to, during and directly after MTX injection increased the severity of mucositis and caused a higher mortality rate. Furthermore, plasma citrulline was increased before MTX injection due to the FXR-agonist. Even more, both plasma citrulline and the villus length increased due to the FXR-agonist in the recovery phase. Therefore, the FXR-agonist probably increased the proliferation, making the intestine more prone to MTX, resulting in increased severity of mucositis. This suggests that the FXR-agonist functions as an intestinal trophic factor, which is in agreement with a study where enterally administered bile salts, which functions as FXR-ligands, increased intestinal growth in total parenteral nutrition associated atrophy in piglets [38,40]. Therefore, we conclude that an FXR-agonist should not be administered directly prior and after MTX injection. However, administering an FXR-agonist as a therapeutic option a few days after MTX to fasten the recovery could be of great interest and further research is needed. Furthermore, since bile salts are endogenous FXR ligands, this suggests that bile salts are of influence in the pathophysiology of mucositis [26-28,41,42]. Therefore, FXR and bile salts are novel target options to alter the severity and recovery of mucositis. Furthermore *chapter 4* shows, although not the main aim and therefore not further outlined, that animals receiving MTX have a significantly different microbiota composition with a decreased diversity, as shown before [43]. Therefore, we conclude that the pathophysiology is not completely elucidated yet, due to the fact that multiple potentially important factors, like bile salts and microbiota, are so far not included in the 5-phase model of mucositis.

CLINICAL CARE OF MUCOSITIS

Risk analysis and diagnosis of mucositis

In c*hapter 5* we reviewed the current literature on risk, diagnosis and management of the symptoms of mucositis. The main focus is the pediatric clinical setting. However, the number of clinical trials performed in pediatric cancer patients was limited. Therefore, in the absence of pediatric clinical trials studies on adults provided indications for use in children. We conclude from *chapter 5* that three steps are important in order to be able to give adequate supportive care in children suffering from mucositis.

The first step is to know the risk factors for developing mucositis. Treatment-related factors like the type of chemotherapeutic agent, the dosage and the dosing schedule all influence the development of mucositis. Furthermore, patient-related factors like a lower body surface area, gender (female), and Caucasian ethnicity, showed in adult studies to be associated with an increased risk to develop mucositis [44-48]. However, no studies have been performed in children. Finally, concerning genetic factors in the treatment of either leukemia or lymphoma, children

with the XRCC1 Arg 399 Gln polymorphism might have an increased risk to develop mucositis [49]. Furthermore, in children receiving MTX treatment the MTHFR C677T polymorphism is a possible risk factor for mucositis [50].

The second step, in order to be able to give adequate supportive care, is to recognize and diagnose mucositis objectively. We analyzed the use of assessment scales in the literature and we conclude that these scales are based on symptoms which are not only caused by mucositis, and thus are non-specific. Furthermore, pain relief medication influences the assessment scales, and finally most assessment scales have not been validated in children [51,52]. Young children are not capable of expressing their feelings, like the level of pain. Therefore, we conclude that assessment scales are subjective and might underestimate the level of mucositis in young children. However, currently almost all adult and pediatric cancer centers use assessment scales to diagnose and assess the severity.

The third step is to give adequate supportive care on symptoms and consequences of mucositis. We conclude that based on data from the current literature the management of pain and diarrhea due to mucositis is still difficult. Unfortunately we have to conclude that no clinical trials have been performed concerning nutritional support during mucositis in either adult or pediatric patients. Overall, the conclusion from *chapter 5* is that the management of pain, diarrhea and nutritional support during mucositis are still challenging and more research is needed. Furthermore, the risk analysis and diagnosis of mucositis are dependent on a diagnostic tool, for which a biomarker would be very useful.

Therefore, in *chapter 6* we gave an update of the studied biomarkers and tests to diagnose mucositis. To diagnose mucositis and to establish the severity of mucositis ideally a biopsy of the small intestine via endoscopy would serve as the gold standard. However, performing an endoscopy in immune compromised patients is invasive, can be painful, and there is a high risk of infection or bleeding. Therefore, this is not an option in patients suffering from chemotherapy-induced mucositis. Since this gold standard is not possible and assessment scales are subjective and might underestimate the severity, we are in need of a biomarker or test to diagnose and establish the severity of mucositis. A biomarker is defined as "a human or animal biological property whose in vitro measurement or identification is useful for the prevention, diagnosis, prognosis, treatment, and follow-up of human or animal diseases, and for their understanding"[53]. Moreover, this biomarker should be easy accessible, non-invasive and sequentially determinable. In our opinion a biomarker should be present in the healthy individuals and should be altered due to mucositis. Therefore, in *chapter 6* we made a division in biomarkers, which are actually present in the body, and tests, which need the administration of any kind of substrate to the patient before measurement. Biomarkers in blood samples, like I-FABP and I-BABP, are potentially interesting since they are markers of enterocyte loss in the small intestine [54]. However, these markers are probably only of value in combination with another biomarker. Furthermore, in blood samples one of the most interesting and studied

biomarker so far is plasma citrulline. It is an amino acid synthesized almost exclusively by the enterocytes of the small intestine, and thereby a marker of the enterocyte mass [55-58]. It correlates with the severity of mucositis as measured by villus length of the jejunum in rats [10]. Moreover, it was shown in clinical studies that plasma citrulline correlates with the severity of mucositis in adults and children [55,56,59-61]. In addition, biomarkers in feces, like calprotectin and calgranulin, are promising to detect intestinal inflammation, but probably not useful in neutropenic patients, therefore possibly only interesting in radiation-induced mucositis [55,62-64]. Besides biomarkers, also the use of non-invasive tests to determine mucositis is promising, like the sucrose breath test, but more clinical trials are needed to draw conclusions [65]. We conclude from *chapter 6* that plasma citrulline is currently one of the most promising biomarkers, and therefore we suggest to use this marker in future clinical trials and animal studies. In this way we will be able to compare studies for the incidence and severity of mucositis in different clinical settings. However, since the pathophysiology of mucositis is so complex, we are possibly in need of a combination of biomarkers to diagnose and assess the severity of mucositis accurately, and we are therefore in need of more research.

Optimizing feeding strategy

One of the major problems during mucositis is the decrease in nutritional intake leading to a deteriorated nutritional status. In pediatric cancer patients a deteriorated nutritional status has led to a delay of chemotherapy cycles and a reduction of chemotherapy dosages [66,67]. Therefore, to be able to tolerate the high dose chemotherapy treatment protocol the child has to stay in a good nutritional condition. To obtain this during mucositis is challenging. From previous experiments in our mucositis rat model we gained knowledge about the feeding strategy during mucositis. In the mucositis rat model Fijlstra et al. have shown that lactose and fatty acids were not digested and absorbed during mucositis even if continuously administered enterally [10,68,69]. In contrast, glucose and amino acids were still absorbed if administered continuously enterally [68,70]. Furthermore, total enteral nutrition was compared with total parenteral nutrition (TPN). The administration of total enteral nutrition in the rat model was not feasible, and TPN was superior to maintain bodyweight in the rat compared to the enteral feeding [71]. However, TPN has disadvantages, like villus atrophy, mucosal permeability, and an increased risk to develop an infection [72-75]. Therefore, in *chapter 7* we aimed to determine the feasibility of administering minimal enteral feeding (MEF) during mucositis in the rat, and thereby determine the effect of MEF on the recovery. We administered only 20% of the normal caloric intake as tube feeding, which is comparable to MEF administered in other intestinal animal models [76-78]. Based on the results from previous experiments we knew that an elementary diet has the best absorption possibility, therefore we administered the most elementary diet available in clinical practice. The results show that the administration of MEF does not increase the morbidity due to mucositis and therefore we concluded that MEF is feasible during mucositis in the rat.

Moreover, the results show a higher food intake and body weight gain, and a significantly longer villus length in the recovery phase, suggesting that the recovery is faster by the administration of MEF during mucositis. Therefore, we conclude from *chapter 7* that MEF is feasible during mucositis and we suggest that MEF accelerates recovery after mucositis.

As discussed above, in clinical practice the optimal feeding strategy during mucositis is unknown. Patients are often unable to eat normally and their own intake decreases drastically. Therefore, most patients are in need of nutritional support. In general, nutritional support in the pediatric cancer patients is challenging, there is no consistency, and both enteral nutrition and TPN have advantages and disadvantages. Tube feeding is not ideally during nausea and vomiting. However, TPN may induce villus atrophy and mucosal permeability, and increases the risk to develop an infection and liver dysfunction [72-74,79,80]. In a systematic review concerning critically ill pediatric patients there was a lack of data and the main conclusion was that research is urgently needed [81]. The question arises, what is the current clinical practice of nutritional support in the pediatric cancer patients suffering from mucositis? In order to answer this question we designed an observational study in three pediatric cancer centers in the Netherlands to observe the feeding strategy as described in *chapter 8*. The results show that one center in the Netherlands administered TPN as standard care after every stem cell transplantation, independent of the nutritional status, and added tube feeding in case of bodyweight loss. This is in contrast to two other centers in the Netherlands, where tube feeding is the first choice and only TPN was added if total tube feeding was not tolerated and the patient was decreasing in bodyweight. Furthermore, the results show that both tube feeding, TPN or a combination of these two were administered. Although it is only an explorative study, the results may give a few suggestions. The results suggest that patients receiving tube feeding alone lost more weight, which suggest that it is preferably to start with TPN to maintain the nutritional status. On the other hand, the data suggest that the patients receiving TPN, either alone or combined with enteral tube feeding, had more episodes with fever and antibiotic treatment, and were longer hospitalized after chemotherapy courses. This suggests that it is preferable to start with enteral tube feeding as first step. However, it is possible that the patients who received TPN were more severely ill, suggested by the lower plasma citrulline levels, which explains the need of TPN and the prolonged hospitalization. These results from the observational study in *chapter 8* show advantages and disadvantages both for tube feeding and TPN. The second focus of *chapter 8* is an online survey concerning, amongst other things, feeding strategies worldwide. The results from the survey, although a small number of centers participated, suggest that there are large inter-center differences in feeding strategy. Tube feeding, TPN or a combination are all first choice as nutritional support. Even more, some centers do not administer tube feeding at all during mucositis, although in the majority of centers minimal enteral feeding is added to TPN if it is feasible. Recently, one study determined the difference between early and late TPN in critically

ill pediatric patients. They showed also that late TPN was superior for, amongst other things, less new infections and a shorter duration of hospital stay [82]. A systematic review concerning nutritional support in children with cancer receiving chemotherapy, not specifically mucositis, suggested that TPN might be superior compared to normal food intake [83]. However, not one study compared tube feeding with TPN and the main conclusion was that further research is essential. Furthermore, in children undergoing hematopoietic stem cell transplantation, not specifically during mucositis, enteral nutrition is suggested to be the first option and parenteral nutrition the second [84].
In conclusion, since there are both discordances in the clinical practice as well as a lack of knowledge we are in need of clinical trials concerning the feeding strategy during mucositis. In the meantime, based on our results in combination with the current literature, we suggest that as first step tube feeding is preferable, with an easy access to TPN. TPN might be superior to maintain weight, but might increase infections and is therefore risky as first step, although more research is needed to draw this conclusion.

FUTURE PERSPECTIVES

Prevention and treatment of mucositis

In this thesis we interfered in three different ways in the pathophysiological model of mucositis. Unfortunately, we can conclude that the interventions presented in this thesis did not lead to a decrease in the severity of mucositis. It is possible that an alteration of one target in this complex model of mucositis is not enough to prevent mucositis, however this should at least decrease the severity at some level. Therefore, the question arises if the currently used 5-phase pathophysiological model is correct for gastrointestinal mucositis. Multiple potentially important factors, like bile salts and microbiota, are so far not included in the model. Therefore, we conclude that the pathophysiology of mucositis is not completely understood. In the search for prevention or treatment of mucositis we suggest that future research should focus on multiple targets, thereby combining both the elucidation of the pathophysiology, and the search for targets to prevent or treat mucositis.

First of all, we suggest the focus of future studies should go back to *the mechanism of mucositis*. What happens in the intestinal mucosa both in the developing stage as well as the recovery stage. We suggest to use the MTX model as first step to reveal the pathophysiology of mucositis. Determine several influencing factors, like inflammation, proliferation and apoptosis, on different time points. Create a timeline to find the influencing factors important at certain time

after chemotherapy. Especially the determination and timing of the inflammatory cytokines should be a focus.

Second, a main focus of future experiments should be the *microbiota*, a large research field which is missing in the current pathophysiological model. As shown in *chapter 4*, the microbiota composition is changed and the diversity is decreased in the mucositis animals, as also previously determined in the MTX rat model [43]. The question is if this change in microbiota is either the cause of mucositis or secondary to mucositis. As already suggested in a few studies, as reviewed by van Vliet et al, the microbiota are probably very important in both the development of mucositis as well as in the recovery of mucositis [85]. Therefore, we suggest to focus future studies on unraveling the relation between the microbiota and mucositis in our mucositis rat model. Elucidate which bacteria contribute to the severity of mucositis. Furthermore, interventions to alter the microbiota, like antibiotics, pre- and probiotics, should be studied in this rat model of mucositis to determine the importance of the microflora. This can then be extended to the clinical practice, although probiotica in immune compromised patients is possibly not without risks.

Third, based on this thesis other targets might be of great interest for future studies. What is the influence of *bile salts* on the pathophysiology of mucositis and on recovery? We showed in this thesis that bile salts are possibly altered and influenced during mucositis. Therefore, we suggest future animal studies should focus on possible interventions in alteration of bile salt composition after chemotherapy in order to fasten the recovery. Furthermore, in this thesis the bile salts were determined in rats, but it is unknown what the effect of chemotherapy on bile salts is in humans. Therefore, future clinical trials should determine the alteration in composition and amount of bile salts during mucositis.

Finally, in this thesis we measured plasma citrulline sequentially to determine the severity of mucositis over time, in order to minimize the number of animals needed in our studies. We suggest future studies should try to decrease animal experiments even more by focusing on other experimental methods to study mucositis. Recently, organoids, three-dimensional in-vitro grown cells, have been a focus as possible new model system in research [86]. Therefore, we suggest to focus on *organoids*, as a possible alternative experimental method to study mucositis.

Clinical care of mucositis

Till there is an intervention to prevent mucositis, future studies should focus on increasing the quality of life during mucositis, by improving the supportive care of symptoms and consequences of mucositis.

First, we suggest to use plasma citrulline as potential biomarker in future clinical trials and animal studies. In this way we will be able to compare studies for the incidence, risk factors and severity of mucositis in different clinical settings. Furthermore, future research should validate the diagnostic accuracy of plasma citrulline in the clinical practice.

Second, an underestimated focus so far is the mental condition of the children influencing the development of mucositis. Stress of isolation instead of group housing influenced mucositis in rats, and in *chapter 7* we showed an increased mucositis due to stress of attachment to the swivel system [87]. But what is the influence of *stress* on the severity and recovery of mucositis in pediatric patients? Therefore, we suggest to include stress and the quality of life as secondary parameters in clinical trials in pediatric cancer patients during mucositis.

Finally, the clinical practice is in need of a *feeding strategy protocol*. In this thesis we showed that it is feasible to administer MEF in the rat during mucositis, and we suggest that MEF increases recovery, but is this also the case in humans? Furthermore, there is no consensus in the clinical practice around the world; the feeding strategy is currently probably based on both patient and doctor preferences. Since this is not based on evidence based medicine, feeding strategy should be the focus of studies concerning the clinical practice of mucositis. Both in adult patients as well as pediatric patients randomized clinical trials should be performed. Currently we are performing a randomized clinical trial in adult haematologic stem cell transplantation patients to compare enteral tube feeding with TPN. Therefore, we suggest as next step to perform a randomized clinical trial with the comparison of tube feeding and TPN as nutritional support in pediatric cancer patients. Furthermore, we suggest to perform a clinical trial to determine the feasibility and effect of MEF as additive to TPN on the recovery of mucositis. Eventually this should lead to an optimal feeding strategy during mucositis and therefore should lead to the development of a guideline with evidence based medicine for feeding strategy in both adult and pediatric cancer patients suffering from mucositis.

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